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(54) METHOD FOR PRODUCING A SPECIFIC ANTISERUM AGAINST THE UNIVERSAL TUMOROUS ANTIGEN AND METHOD FOR DIAGNOSING MALIGNANT TUMOURS USING SAID ANTISERUM

(57) The present invention pertains to the field of medicine and may be used for producing a specific antiserum as well as for carrying out immunological diagnoses of malignant tumours. This method for producing an antiserum involves sampling an embryo at the foetal stage from animals of a same genetic type so as to obtain a cell suspension. After immunisation, this method involves sampling spleen cells from the animal, separating lymphocytes and immunising the animal of the same genetic line using the lymphocyte suspension. An antiserum is then obtained and cells originating from healthy organs of the same animals are added to said antiserum. The mixture is finally decanted and the liquid located above the sediments is filtered. In order to carry out a diagnosis, the filtrate is added to the subject's blood and the results are obtained by immuno-fluorescence, by blood tests or using other methods of immunological diagnosis. It it thus possible to diagnose a tumour when the reliable values obtained differ from reference values.

EP 1 072 272 A1

Description

FIELD OF INVENTION

[0001] The present invention pertains to the field of medicine, particularly to oncology, its spheres and diagnosing malignant tumors.

BACKGROUND OF INVENTION

[0002] A brief review of immunodiagnosis in the oncology shows the following.

[0003] In 1949 it was first mentioned by L.A. Zilber and in 1957 it was proved by T. Pran and G. Main that malignant cells have their own antigens.

[0004] According to Abilev there are 4 groups of antigens.

1) Viral tumorous antigens. They are identical for any viral tumor of this type.
2) Carcinogenic tumorous antigens. They are individual for patients as well as for tumors.
3) Isoantigens of transplantation type or tumorous-specific transplantation antigens. They are different in all individual types of tumors, inducted by chemical agents. And they are the same in different tumors caused by the same virus.
4) Embryonic antigens.

[0005] During the process of carcinogenesis, cells are put to dedifferentiation, thus they acquire an embrional structure. In them there are to be found embryonic antigens, specific to embryonic development of organisms. These antigens can immunize the organism against tumors. The more studied antigens are the following: α-fetoprotein and cancer embryonic antigen (CEA). The former is to be found by carcinoma of the liver, the latter - by adenocarcinmoma of the intestine, stomach, esophagus and pancreas.

[0006] Children having neuroblastoma, lymphosarcoma or tumor of the brain have α_2-fetoprotein. Those who have carcinoma of the stomach have - fetal sulfoglycoprotein. The above mentioned antigens are localized inside the cell membrane or circulate in the blood.

[0007] There is a specific group of antigens, so called heterospecific antigens, existing. They could not be classified as heterologous to the organism, while besides tumors they exist in other normal tissues. Among heterospecific antigens there is a renal antigen, which exists as a norm in the kidney and in the tumor of liver - hepotoma.

[0008] Adenocarcinoma of kidney contains an antigen of lungs and liver.

[0009] The immunological diagnosis of malignant tumors based on indication in the subject's blood the above mentioned antigens, antibodies to them and on revealing sensitized to tumoral antigen lymfocytes.

[0010] Methods for diagnosing lymphosarcoma, neuroblastoma are based on revealing α-fetoprotein. (On revealing antibodies to CEA - see Method in the Patent RU, 2077725, G. 01 N 33/53).

[0011] On revealing heterogenous antigens see Patent RU N 2063768, 1991, A 61K 39/00, Patent RU N 2025734, MPK G 01 N 33/53, author's certificate USSR N 170922, author's certificate N 1589215.

[0012] In the author's certificate N 1805392 (G 01 N 33/53) the method for diagnosing cancer by lymphocyte antigens (H_{la-b} 35) is described.

[0013] In fact, no test of the existing level of diagnosis is universal. Revealing antibodies in blood is a less reliable test, for in human blood there is a very wide spectrum of antitumoral and tissue antibodies. There is no method existing for revealing specific universal tumors antigen.

[0014] In this field perspective is revealing sensitized lymphocytes which inhibit the growth of malignant cells. Though, they are active only against "their own" type of tumor.

[0015] Thus, the used methods for tumor immunological diagnosis do not satisfy in all respects the requirements of primary diagnosis of tumors, they are totally unsatisfactory in screening malignant neoplasms and groups of high risk. Therefore they may be used to a certain degree only in immunomonitoring healing of malignant tumors.

[0016] Failures in existing methods may be explained by the fact that the used antiserums against malignant tumorous antigens do not satisfy their own characteristics.

REVEALATION OF THE INVENTION

[0017] The aim of the Invention is producing antiserum against universal tumorous antigen, independent of tumor and organ type.

[0018] The prototype of the claimed method for producing specific antiserum as well as the method for diagnosis using the said antiserum is the method described in Patent RU N2063768, 1991, IPC, A 61 K 39/00. This method

involves sampling tumor tissues from dead men, its freezing, obtaining a cell suspension, cell dispersion and decantation, extraction of antigens from supernatant fluid, extract immunization of animals, sampling blood from the immunized animals, obtaining the product from it, filling the specific antiserum into the reaction with the subject's blood, on the result of which a tumor is being diagnosed.

[0019] The claimed method unlike the well-known one allows obtaining antiserum for idotype of the T - cellular receptor functioning in malignant tumors. That is to obtain antiidotypic antiembrionic serum. That allows diagnosis of all types of tumors independently of their genesis and situs.

[0020] To accomplish the method for producing specific antiserum it is necessary to carry out two-stage immunization. That involves sampling an embryo at the foetal stage from animals of the same genetic type, dispersing it, obtaining cell suspension. Then the animal of the same genetic line is immunized by the cell suspension. After immunization it is necessary to sample spleen cells from the animal, to separate lymphocytes from the cell suspension at density gradient of 1,065-1,079. Using the above-mentioned lymphocytes it is necessary to accomplish multiple immunization of syngenic intact animals and then obtain antiserum from them using the standard method. This antiserum should be filtered (approximate diameter of pores in filters is 20 mcm).

[0021] The obtained antiserum has given reaction of precipitation with different types of tumors sampled from different people and in different organs.

[0022] It allowed devising methods for diagnosing malignant tumors on the basis of the obtained antiserum.

[0023] The well-known analogous methods for diagnosing tumors possess not high enough sensibility. Even the more effective among them have the sensibility level of not more than 40-60%. Such a low level of well-known oncologic immunodiagnostic tests can be explained so that the oncomarkers, used in said reactions, do not in fact satisfy their own characteristics. They are organo-specific or oncofetal antigens, characteristic of individual organisms or systems of organs in a norm. It leads to the following; an expected universal immunological expression of the only tumorformation mechanism pecularities is substituted by individual, characteristic of not tumoral conditions (inflammation, collagenosis).

[0024] Organo-specific antigens are well-known for being not binding for tumoral cell-transformation. That gives high percent of pseudopositive results by diagnosing malignant tumors.

[0025] The proposed method for revealing of the oncomarker strongly differs from the well-known ones by disclosing a universal highly specific antigenic marker of tumoral growth, preserving during the whole period of tumoral progression.

[0026] The method is based on the results of author's theoretical and experimental works establishing that in histologically different cells of malignant tumors there is a stable in tumoral progression process functioning, which recognizes superficial embryospecific antigens. The said mechanism is proved to be on the basis of tumor formation (immortalization and progress) phenomena.

[0027] To carry out the method for diagnosing tumors it is necessary to obtain the specific antiserum using the proposed method, to fill the antiserum against universal tumoral antigen into immunological reaction with the subject's tissues or physiologic fluid. After it the tumor is diagnosed by immuno-fluorescence or by blood tests. Tissues of tumor in immuno-fluorescence reaction or the subject's blood test can be used as tissues.

[0028] The tumor diagnosis is proved by statistically reliable differences of the reaction results between tentative and control tests.

[0029] By obtaining the blood test the following formula for calculating the differences between tentative and control tests should be used:

$$\alpha = \frac{\left| \left(A - \frac{B_1 + B_2}{2} \right) \right| \times X}{50}$$

where

α - diagnostic coefficient, if there is a tumor $\alpha \geq 1,5$
A - definition of blood test in tentative test (the antiserum for tumor antigen is added to the subject's citrated blood)
B_1 and B_2 - definition of the blood test in control tests (the serum of the same animal, used for obtaining the antiserum, is added to the subject's citrated blood)
X - maxim definition of the blood test in the test
A or the average B_1 and B_2 that is

$$\frac{B_1 + B_2}{2}$$

VARIANTS OF INVENTION ACCOMPLISHMENT

[0030] An example of accomplishing methods for diagnosing malignant tumors.
At the foetal stage an embryo is sampled from Wister line rats, of 300 - 500g. Weight. The embryo's tissues are dispersed in the medium 199 at the following correlation of volumes and tissue: medium 199 1:5. The obtained suspension is used for weekly immunization of the intact Wister line rats.
After a period of 1,5 month spleen cells are taken from the animals, dispersed and at the phyco-verografin gradient 1.065 - 1.079 lymphocytes are obtained.
From the said lymphocytes the suspension is obtained: medium 199 at the correlation 1;1, which is weekly put into other intact rats. After five immunizations the rats are killed, the blood is taken from them, "lightened", the antiserum is obtained from it, filtered. With the above mentioned antiserum the blood test was taken from the below mentioned groups of patients. To carry out the blood test the standard capillary with innerdiameter about 0,8mm is used. 800 mcl of whole fresh venous blood, taken during the analysis, not late then 20 seconds after the moment of taking, is added to 200 mcl of 5% - sodium citrate bufer solution. The analysis should be carried out within 60 minutes since mixing the blood and the preservative. The analysis must not be taken in the case of hemolysis or coagulation. The said blood should be shared into 3 parts, 70mcl each, the parts should be put into three separate test-tubes. One of the parts should be added with a tentative (with antibodies) serum, the two others should be added with a control (without antibodies) serum, 20 mcl each. The serums are to be filled directly into the blood with preservative, not on the walls. The capillaries should be of the same size. The mixtures are mixed and filled into the capillaries till the level mark 5/0. This way they are to be kept 60 minutes. 60 Minutes later the indices are to be read and calculated according to the above

mentioned mathematic formula.

[0031] The antiserum, obtained by the said method using Wistar line rats, was used for diagnosing disease with certain patients.

In the blood test of a patient K., 1942 y.o.,

d-s: rectum carcinoma, the obtained results were the following:

$A=25$, $B_1=28$, $B_2=28$

According to the mathematic formula the coefficient α was calculated:

$$\alpha = \frac{\left| \left(25 - \frac{28+28}{2}\right) \right| \times 28}{50} = 1,7$$

1,7> 1,5, that is diagnosis malignant tumor was proved.

[0032] In the blood test of a patient with fibroma of lobule of the auricle the results were the following:

$A=10$, $B_1=12$, $B_2=12$

According to the mathematic formula the α coefficient was calculated:

$$\alpha = \frac{\left| \left(10 - \frac{12+12}{2}\right) \right| \times 12}{50} = 0{,}48$$

$\alpha < 1{,}5$, that is diagnosis of benign tumor was proved.

[0033] Below there are the results of investigating a group of patients having malignant tumors.

Comedocarcinoma - 125 patients
Sensibility - 83,2%
Carcinoma of the lung - 247 patients
Sensibility - 98,1%
Carcinoma of the stomach - 156 patients
Sensibility - 85,2%
Carcinoma of the colon intestine - 23 patients
Sensibility - 82,5%
Carcinoma of the rectum intestine 27 patients
Sensibility - 92,5%
Struma maligna - 58% patients
Sensibility - 79,5%
Carcinoma of the kidney - 38 patients
Sensibility - 78,6%
Carcinoma of the body of the womb - 412 patients
Sensibility - 75,0%
Carcinoma of the neck of the womb - 41 patients
Sensibility - 81,8%

Control group

[0034]

Almost healthy - 400 patients
Sensibility - 5,1%
Mastopathia cystica-fibrotic - 221 patients
Sensibility - 8,3%
Gastritis - 120 patients
Sensibility - 6,2 %
Gastric ulcer - 62 patients
Sensibility - 8,3%

Collagenosis - 40 patients,
Sensibility - 6,5%
Pneumonia - 40 patients,
Sensibility 7,2%
(acute and chronic)
Prostatitis - 18 patients,
Sensibility - 2,1%
Chronic colitis - 115 patients,
Sensibility - 4,2%

INDUSTRIAL APPLICABILITY

[0035] Conclusion: The proposed method possesses sensibility not less than 92,4%, it is a highly effective diagnosing test.

[0036] In the application specific antiserum against universal tumoral antigen is the main component of the diagnosing device, on sale in Russia and abroad under the trade mark Turtest [R]

Claims

1. The method for producing specific antiserum for a universal tumoral antigen, involving sampling tissues, obtaining cellular suspension, immunization of animals, sampling blood from the immunized animals, obtaining the claimed product from it, characterized by multiple immunization, at the first stage as tissues an embryo at foetal stage is sampled from animals of the same genetic type so as to obtain a cell suspension, after immunization sampling spleen cells from the animal is carried out and lymphocytes are separated from the suspension, the subsequent immunizations of the animals of the same genetic types are carried out using the lymphocyte suspension, an antiserum is then obtained from the animal and cells of intact organs of the same animals are added, the mixture is decanted and the liquid located above the sediments is filtered.

2. A method as claimed in Claim 1, characterized in that filtration being carried out through porous filters.

3. Method for diagnosing malignant tumors using a specific antiserum against an universal tumoral antigen, involving sampling tissues, obtaining cell suspensions, immunizing animals, obtaining antiserum, filling it into reaction with blood or other physiologic liquids of the subject, on the results of this reaction a tumor is diagnosed, characterized by that the multiple immunization is carried out, as tissues at the first stage an embryo at foetal stage is sampled from animals of a same genetic type so as to obtain a cell suspension, after immunization sampling spleen cells from the animal is carried out, lymphocytes are separated from the suspension, the subsequent immunizations of the animals of the same genetic type are carried out using the lymphocyte suspension, an antiserum is then obtained from the animal, added to tissues, blood or other physiologic liquids of the subject with the following reading if the results by immuno-fluorescence, blood tests or other well-known methods for immunodetection, a tumor is then diagnosed by indices different from the control indices.

4. A method as claimed in Claim 3 characterized in that results of the blood test are calculated by the formula:

$$\alpha = \frac{\left| \left(A - \frac{B_1 + B_2}{2} \right) \right| \times X}{50}$$

where:

α - diagnosing coefficient, if there is a tumor it makes ≥ 1,5
A - index of the blood test in the tentative test (an antiserum against tumor antigen is added to the subject's blood)
B_1 and B_2 - index of the blood test in control tests (the serum of the same genetic type of animal, used for antiserum producing, is added to the subject's blood)
x- maximum index of the blood test in the analysis

(or in the test A or average B_1 and B_2, that is $\frac{B1 + B2}{2}$).

INTERNATIONAL SEARCH REPORT

International application No.
PCT / RU 98/ 00143

A. CLASSIFICATION OF SUBJECT MATTER [6]:
IPC6: A61K 39/395, G01N 33/531

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC6: A61K 39/395, G01N 33/48, 33/487-33/493, 33/53, 33/531

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RU 2009502 C1 (FIGURNOV VALENTIN ALEXANDROVICH) 15 March 1994 (15.03.94), column 4 of the description	1-2
A	RU 2025734 C1 (EI-EM-DI-EL, INC.) 30 December 1994 (30.12.94), the abstract	3-4
A	WO 83/04102 A1 (PARAGON DIAGNOSTICS) 24 November 1983 (24.11.83), the abstract	3-4
A	EP 0335804 A1 (INSTITUT MERIEUX) 04 October 1989 (04.10.89), the abstract, the claims	1-2,3-4
A	EP 0453082 A1 (HYBRITECH INCORPORATED) 23 October 1991 (23.10.91), the abstract	1-2
A	US 0453082 A (HYBRITECH INCORPORATED) 28 December 1993 (28.12.93), the abstract	1-2

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"&" document member of the same patent family

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